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MICROCALORIMETRIC STUDY OF YEAST GROWTH, UTILIZATION OF DIFFERENT CARBOHYDRATES*

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ABSTRACT

The anaerobic growth of the yeast *Saccharomyces cerevisiae* on six different mono- and disaccharides was investigated calorimetrically. From the thermograms the kinetic data for the sugar uptake were determined. This methods holds for biphasic growth (diauxy) on mixed sugars too.

INTRODUCTION

Calorimetry of growing organisms is usually very unspecific, because the calorimetric signal represents the sum of the momentary heat production arising from the anabolic and the catabolic processes in the organism. Owing to the complexity and multiplicity of cellular metabolism, many different thermograms are obtained, which can be only inadequately interpreted[1]. But nearly always the physiological and outer parameters may be chosen in such a manner that a simpler, controllable metabolism takes place. This condition holds if one of the components of the nutrient medium is growth limiting. With microorganisms, it is possible to follow calorimetrically the utilization of an energy substrate (e.g., carbohydrates), i.e., the rate of degradation, turnover and energetic efficiency.

If two different carbohydrates are present in the medium diauxy is possible, i.e., a stepwise uptake of the carbohydrates. Diauxy occurs if the organism possesses constitutive enzymes for the first carbohydrate but requires induced enzyme synthesis before it can use the second one.

MATERIAL AND METHODS

A diploid wild strain of the yeast *Saccharomyces cerevisiae* was used in all experiments. The growth medium was prepared from yeast extract, peptone and sugar in a concentration of 4g l^{-1}. The saccharides were D-glucose, D-fructose,

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D-mannose, D-galactose; the disaccharides were sucrose and maltose. For the diauxy experiments, only two of the different sugars were mixed in the growth medium.

The heat production of the anaerobic batch cultures was determined in a Calvet microcalorimeter (type MS 70 from Setaram/France with 100 ml vessels). The cultures were kept at 30°C and stirred. The aerobic batch culture grew in a 1l fermentor provided with oxygen by intensive stirring and air bubbling. A small portion of the volume was continuously pumped through a flow microcalorimeter (Type 10700-1 from LKB/Sweden with a working volume of 0.5 ml). A detailed description is given by Lamprecht[2] and Brettel[3].

GROWTH ON ONE CARBOHYDRATE

If the anaerobic growth of micro-organisms in a liquid medium with sufficient salts, vitamins and sugar as energy source is studied calorimetrically smooth, asymmetric thermograms are obtained (Fig. 1). After an initial lag phase, due to adaptation of the organisms to the medium, the heat production increases according to the exponential growth of the culture. Since in this case the carbohydrate (glucose) is the only primary energy source, its concentration decreases rapidly by degradation of the glucose via glycolysis to ethanol. The maximum heat flux is reached at a residual glucose concentration of approximately 1g l^{-1}, independent of the initial concentration, and drops to zero when the glucose is exhausted. If the culture has a sufficient supply of oxygen, the ethanol accumulated during glucose fermentation can be respired in a second growth phase (Fig. 2). In our case, the diauxy results from the presence of a second carbohydrate (ethanol), which is produced as a metabolite of the primary sugar, and can only be utilized in the presence of oxygen.

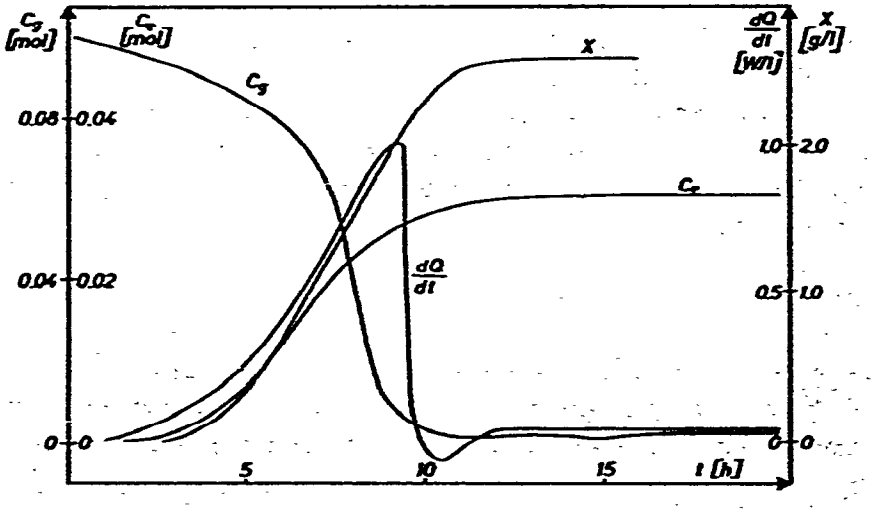

Fig. 1. Thermogram of a growing yeast culture on glucose medium. dQ/dt = heat production; X = dry weight; C_g = glucose concentration; C_e = ethanol concentration.

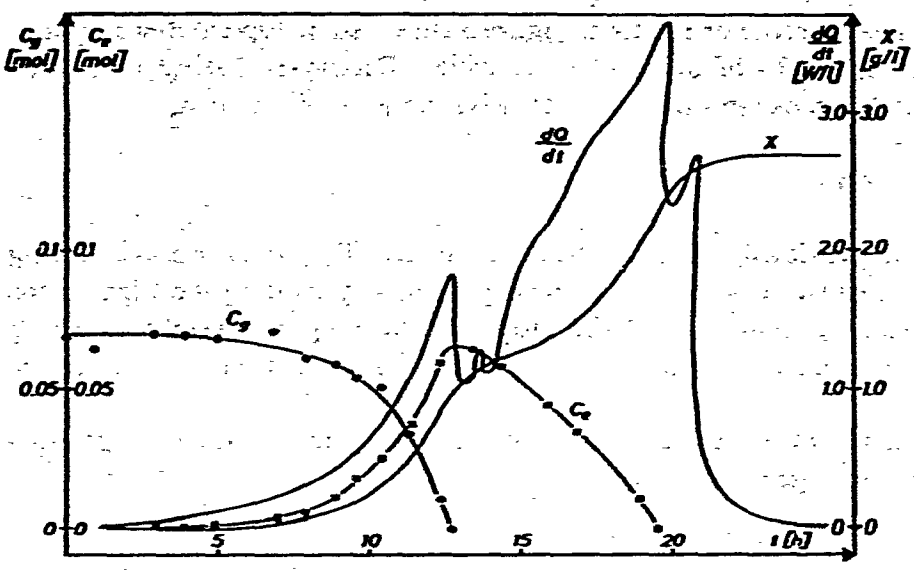

Fig. 2. Thermogram of a growing yeast culture on glucose medium under mainly aerobic conditions (personal communication of R. Brettel/Berlin).

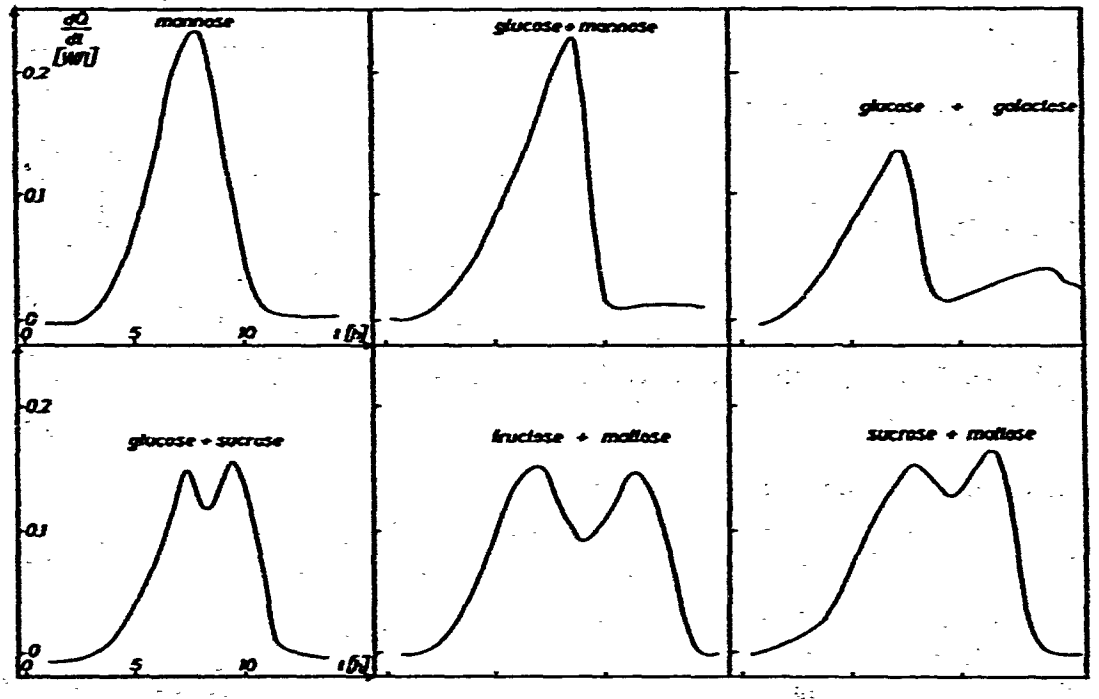

Fig. 3. Thermograms of a growing yeast culture on different carbohydrates.

GROWTH ON TWO CARBOHYDRATES

Diauxy also occurs under anaerobic conditions if the medium contains two different carbohydrates which are taken up and degraded by different enzyme systems. With the different sugars used in these experiments, diauxy occurs whenever one of the

two sugars is galactose, sucrose or maltose. Figure 3 shows some typical thermograms of yeast growth on mixtures of different saccharides. Diauxy is distinguishable as double peaks in the thermograms and as a stepwise increase of biomass.

KINETICS OF SUGAR UPTAKE

It was shown previously that Michaelian kinetics for sugar uptake during growth can be obtained from one calorimetric experiment, providing the sugar is the energy limiting factor for growth, and the growth yield Y [g dry weight of cells/g sugar consumed] and the enthalpy change K [J/g sugar] are constant throughout the culture[4-6].

Under these assumptions the evolved heat quantity Q at any time t is proportional to the consumed sugar $(S_0 - S)$:

$$Q(t) = K \cdot (S_0 - S) \cdot V \tag{1}$$

S = substrate [g/l], S_0 = initial concentration of S, V = volume of calorimetric vessel, and the heat flux is

$$\frac{dQ}{dt} = K \cdot \frac{dS}{dt} \cdot V \tag{2}$$

Because of the increasing biomass

$$m(t) = Y \cdot (S_0 - S) \tag{3}$$

eqn (2) must be corrected to

$$\frac{dQ}{dt} = K \cdot V \cdot \frac{dS}{dt} \cdot m(t) \tag{4}$$

Putting the total heat quantity at the end of growth T

$$Q_T = K \cdot V \cdot S_0 \tag{5}$$

we get from eqns (1) and (4):

$$S = \frac{Q_T - Q}{Q_T} \cdot S_0 \tag{6a}$$

and

$$v = \frac{dS}{dt} = \frac{1}{Y} \cdot \frac{1}{Q_T} \cdot \frac{dQ}{dt} \tag{6b}$$

For the evaluation of eqns (6a) and (6b), only the last part of the thermogram with the decrease of the heat production from its maximum to the zero line is necessary, because it is only during this phase that the glucose concentration decreases significantly and represents the initial part of the kinetics. Figure 4 shows the kinetics and the Lineweaver-Burk plot for a monophasic and a biphasic sugar uptake, from which

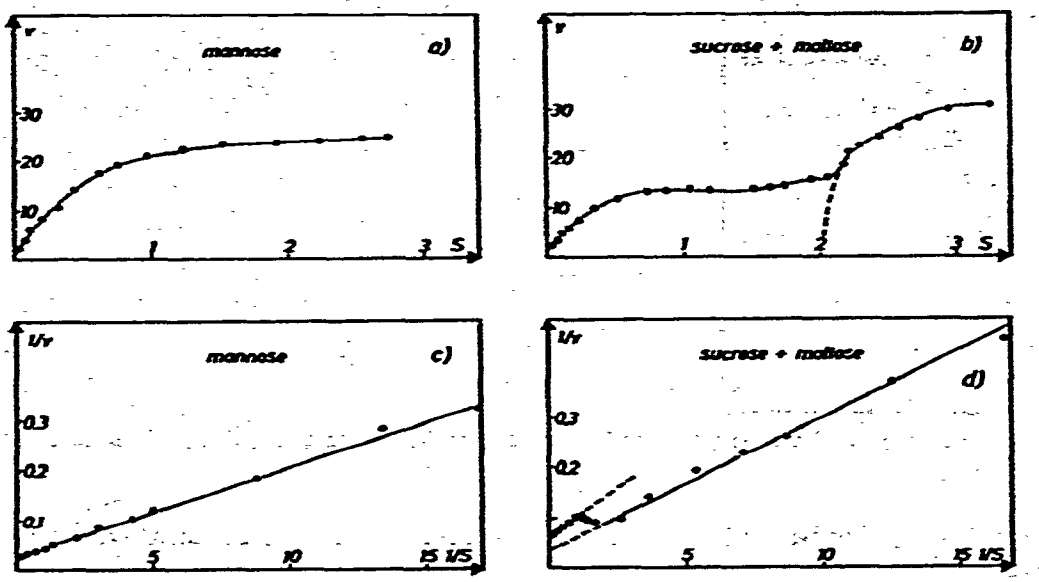

Fig. 4. Michaelis–Menten kinetic (a and b) and Lineweaver–Burk plot (c and d) for a single and a mixed carbohydrate. S is given in (g sugar/l) and v in (mg sugar/min/g biomass).

TABLE 1

K_m AND $v_{max.}$ FOR THE TRANSPORT SYSTEMS OF DIFFERENT SUGARS IN YEAST CELLS

	K_m [g l^{-1}]	$v_{max.}$ [mg min^{-1} g^{-1} biomass]
glucose	0.32	44
fructose	0.76	50
mannose	0.62	32
galactose	0.22	26
sucrose	0.59	40
maltose	1.38	36

the K_m and v_{max} parameters in the model of the Michaelis–Menten kinetics for the transport system can be deduced.

Table 1 shows some preliminary results determined by this method.

These values are derived from the monophasic and diauxic curves and confirm the following features of sugar uptake, already known from genetic and biochemical investigations[7]:

the hexoses glucose, fructose and mannose use the same constitutive transport system;

galactose metabolism involves an inducible transport in addition to inducible enzymes for degradation, and it requires time for the induction;

sucrose is hydrolysed by invertase outside the cell.

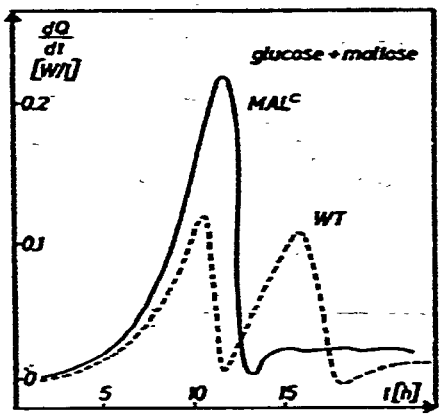

Fig. 5. Thermograms of a wild type (WT) and maltose constitutive mutant (MAL^c) in a glucose/maltose medium.

The resulting glucose and fructose are then taken up by the constitutive transport system,

maltose requires an induction time for transport and for hydrolysis into two glucose molecules.

In the last case, the kinetics are not quite clear, since the Michaelis plots are S-shaped.

ADVANTAGE OF CALORIMETRY

It was pointed out above that only one thermogram is necessary for determining the kinetic data of sugar uptake. Therefore, this simple method is useful for the rapid investigation of different strains or mutants of an organism.

Figure 5 demonstrates such a test with a yeast mutant constitutive for maltose utilization, it is compared with the wild type which needs an induction period for the maltose system as shown by the second peak.

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